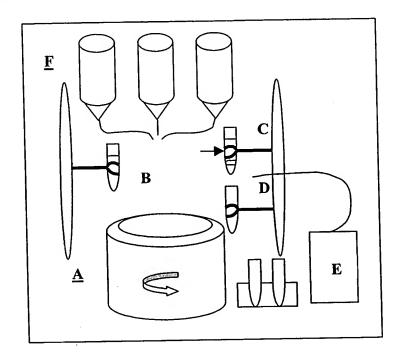
Figure 1: Novel Gene Sequence Analysis Processed cDNA Serial No. To Be Assigned sequence Docket: 506612000100 Reference 1 of 10 (see Example 3) One or more significant hits BLAST UniGene No significant hits One or more significant hits Gene Identified BLAST db_EST Cluster using No significant hits CAP2 One or more Mitochondrial gene, significant hits vector DNA, BLAST nr nonhuman gene, etc. No significant Clone remains hits unknown Unnamed genetic element **BLAST Human** No significant hits Genome One or more significant hits Download contig that contains unknown Take 100kb on Known each side of locus Protein 1. Sequence similarity to known proteins. Unknown 2. Secondary and Tertiary Protein Protein Structure Predict Analysis 3. Domain identification exons 4. Motif identification **Predict** 5. Transmembrane regions peptide 6. Antigenicity profile sequence 7. Hydropathy profile

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Figure 2. Automated Mononuclear Cell RNA Isolation Device



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Figure 3: Kits for discovery of, or application of diagnostic gene sets

A. Contents of kit for discovery of diagnostic gene sets

- 1. Sterile, endotoxin and RNAse free blood collection tubes (>10cc capacity)
- 2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
- 3. Erythrocyte lysis buffer
- 4. Leukocyte lysis buffer
- 5. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence cDNA microarray expression profiling:

Reverse transcriptase and 10x RT buffer

Poly-dT primer

DTT

Deoxynucleotides 100mM each

RNAse inhibitor

Cy3 and Cy5 labeled deoxynucleotides

- 6. cDNA microarrays containing candidate gene libraries
- 7. Cover slips for slides
- 8. hybridization chambers
- 9. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set.

Software facilitates access to and data analysis by centrally located database

- 10. Password and account number to access central database server.
- 11. Kit User Manual

B. Contents of kit for application of diagnostic gene sets

- 1. Sterile, endotoxin and RNAse free blood collection tubes (>10cc capacity)
- 2. Alcohol swabs, tourniquet, 18g needle and syringe (>10cc capacity)
- 3. Erythrocyte lysis buffer
- 4. Leukocyte lysis buffer
- 5. Substrates for labeling of RNA (may vary for various expression profiling techniques)

For fluorescence cDNA microarray expression profiling:

Reverse transcriptase and 10x RT buffer

Poly-dT primer

DTT

Deoxynucleotides 100mM each

RNAse inhibitor

Cy3 and Cy5 labeled deoxynucleotides

- 6. cDNA microarrays containing diagnostic gene sets
- 7. cover slips for slides
- 8. hybridization chambers
- 9. Software package for identification of diagnostic gene set from data

Contains statistical methods.

Allows alteration in desired sensitivity and specificity of gene set.

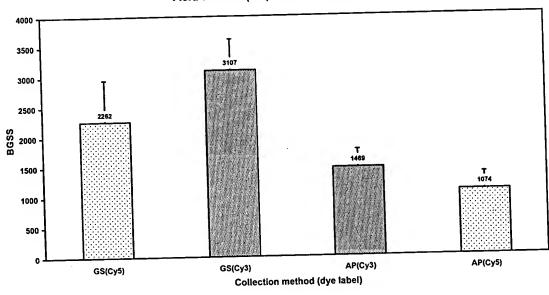
Software facilitates access to and data analysis by centrally located database server

- 10. Password and account number to access central database server.
- 11. Kit User Manual

Figure 4

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Comparison of Guanine-Silica (GS) to Acid-Phenol (AP) RNA Purification



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Expression of Leukocyte Specific Genes

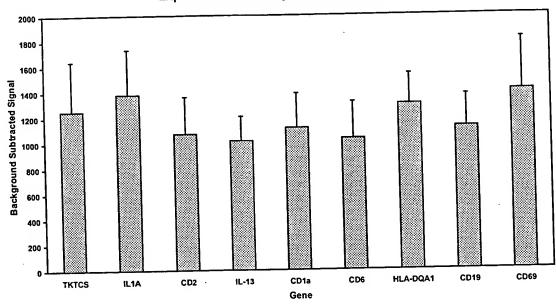
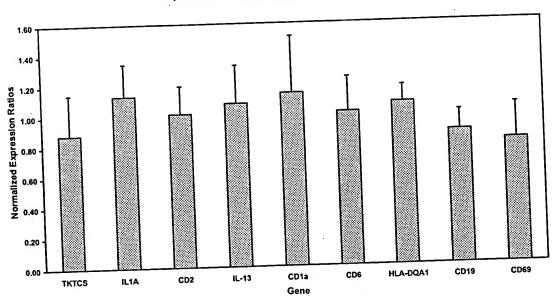


Figure 5

Figure 6

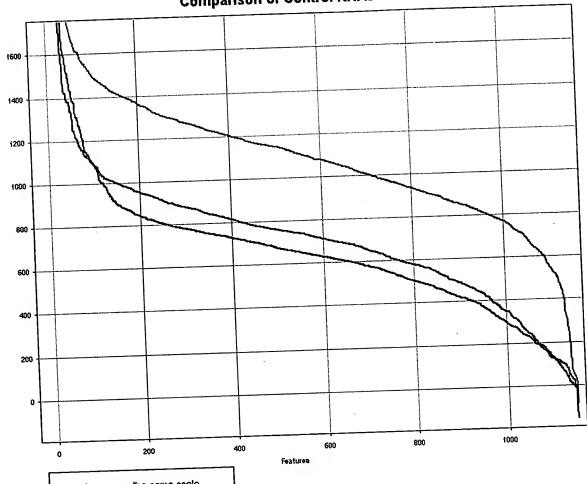
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Expression of Leukocyte-Specific Genes



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Comparison of Control RNAs



All columns use the same scale.

Mononuclear cells, resting and stimulated

-10 Buffy Coats, resting

Mononuclear cells, resting

All markers are connected and ordered by Features.

10 µg of each control RNA was labeled.

Figure 7

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Figure 8: Log expression of each probe using the R50 reference RNA. Probe expression is ordered by Signal to noise, S/N, decreasing from left to right.

Array Hybe 115018

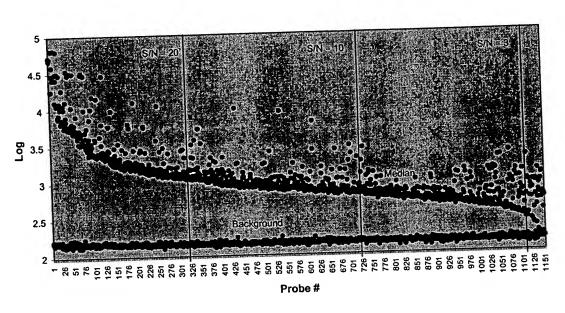
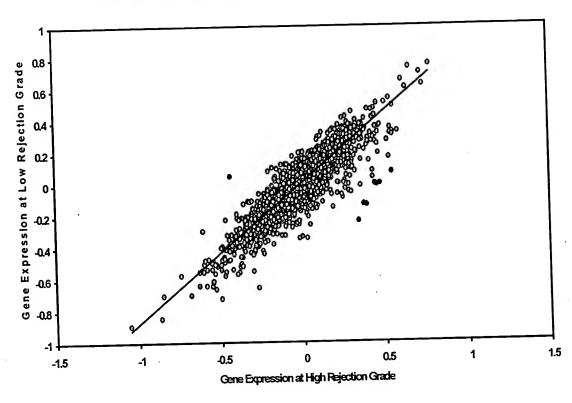


Figure 9

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Comparison of High Rejection Grade to Low Rejection Grade



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Figure 10: Differential gene expression between grade 0 and 3A samples:

							Arrow	107739	· Grade	3Δ	Ratio of	SRs
Probe				07742	Grade	<u> </u>	Array	107739	. Grade	, 37	itatio o.	
				F5 <u>32</u>		SR:	F633	F532		SR:		
					Cy3/Cy5	scaled	Median -	Median -	Cy3/Cy5	<u>scaled</u>		C 2010
	Olige	- 1-		B532	Ratio	ratio (g/r)	B633	B532	Ratio	ratio (g/r)	Grade 0/3A	0.30955069
cc#		2476	5558	1050	0.188917	0.710038	5827			0.219793	3.23048873 3.14462275	0.30933003
IM_003202	transcription factor 7 (T-cell specific, HMG-box) (TCF7),	6025	1810	635	0.350829	1.318579	2150		0.117209			
E220959	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1402	487	0.347361	1.305545	212			0.416612 0.142415		0.3206840
E220959	major histocompatibility complex, class II, DQ beta 1 (HL major histocompatibility complex, class II, DQ beta 1 (HL	2407	804	95		0.444098				0.142413		
1M_002922	regulator of G-protein signalling 1 (RGS1), mRNA /cds= CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	4121	405	0.098277	0.369371	738			0.125045		
IM_001781	lymphotoxin beta (TNF superfamily, member 3) (LTB), tr	2283	13488	3447		0.960516				0.436591		
M_002341	major histocompatibility complex, class II, DQ beta 1 (HL	6025	1539	515		1.257707					2.87796556	
3E220959	CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	3850	386		0.376823		:			2.83369583	
NM_001781	far upstream element (FUSE) binding protein 1 (FUBP1	3581	4507			0.933154		•			2.82568319	
J05040	nuclear receptor subfamily 4, group A, member 2 (NR4A	3729	1365			0.45982					2.80405488	
X14908	transcription factor 7 (T-cell specific, HMG-box) (TCF7).	2476	2716			0.67253		•			2.75642938	0.3627881
NM_003202	cytokine-inducible inhibitor of signalling type 1b mRNA,	642	9850			1 2.00477		•	6 0.04125	4 0.14758	2.70062225	0.3702850
AF035947	CD69 antigen (p60, early T-cell activation antigen) (CD6	2192	. 3357	356	0.10604	7 0.39857	390	S 27	0 0.01120	3 /		
NM=001781	CD69 antigen (pcc, can) . com											
										1.		
	· · · · · · · · · · · · · · · · · · ·			0.4	0.17841	7 0.67057	6 650	576	7 0.87898	2 3.14452	7 0.21325167	4.6892949
Y14737	mRNA for immunoglobulin lambda heavy chain /cds=(65	4905				4 0.64523						
Y13737	mona for immunoglobulin lambda heavy chain /cds=(65	4905		-					8 0.84022	9 3.00588		
BC006402	mona for immunoglobulin lambda heavy chain /cds=(65	4481				1 0.43113	-1		30 0.64752			
X57812	reamment immunoglobulin lambda light chain mRNA /C	3761		-	/ U.114/1	8 0.42176			36 0.64663			
X57812	rearranged immunoglobulin lambda light chain mRNA /C	3761	1	•	0.11221 0 0.112850	0.52086	9 173		92 0.80198	6 2.86907		
X72475	CONA- EL 121321 fis clone COL02335, highly similar to	3790		_	D 0.1360	5 0.51473	-		45 0.8076	7 2.88943		5 5.613396
X72475	CDNA: FL.121321 fis. clone COL02335, highly similar to	3790			-	5 0.48903	- 1		61 0.7732	9 2.76644		9 5.656966
X72475	CONA: FL.121321 fis. clone COL02335, highly similar to	3791	1		-		1					
X57812	reagranged immunoglobulin lambda light chain mKNA /C	3761						33 143	34 0.8175	44 2.9247		
X72475	CDNA: FL 121321 fis. clone COL02335, highly similar to	3791						74 138				
X72475	CDNA: FL 121321 fis. clone COL02335, highly similar to	3790	1		-	• • • • • •						
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4399	-				1	68 185				
X72475	CDNA: EL 121321 fis. clone COL02335, highly similar to	3791 4399	1					74 193	69 0.639	79 2.2888	26 0.1517197	
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	4399	- 4			05 0.3158	99 36			62 2.1701	63 0.1455648	
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	447	- 1			74 0.3410				99 2.3918		
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	1	-	80 0.0900	48 0.3384	44 4		75 0.6856			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	.1			44 0.3181		·-·	0.7080		1	
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	1	-	51 0.0947	03 0.3559	38 1		275 0.8034			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	-1	-	23 0.0920	13 0.3458	28	•••	682 0.7830			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	-		30 0.0982	277 0.369	37 1	0.70	890 0.848			
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	"		33 0.08	396 0.3367				776 2.7818		
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	447	-			363 0.3114			344 0.793	388 2.838		
BC002963	rearranged immunoglobulin mRNA for mu heavy chain e	439	-			103 0.304				313 2.9099 991 3.180		
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439				295 0.311			597 0.888			
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	439			47 0.072	553 0.272	389 16	180 14	148 0.874	413 3.128	101] 0.00/1/1	05 11.4710
AF067420	SNC73 protein (SNC73) mRNA, complete cds /cds=(39	700										